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REVIEW ARTICLE

Effort on planting and product development of *Azadirachta indica* in Southwest China

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Abstract: Twenty-four provenances of two species (*Azadirachta siamensis* and *Azadirachta indica*) have been introduced to China from South Asia, Southeast Asia and Africa Since 1995. This paper summarizes the researches on the introduction and planting of *Azadirachta indica* and analyzes the morphological, phenological characteristics, the growth rhythm, pollinating and seed yielding features of the introduced 24 provenances of the two species as well as the variations of filial generation plants. The experiments showed that most of the provenances of *A. indica* have normal growth and can blossom and fruit in the dry-hot valleys with tropical climate conditions in Yunnan Province, China. The normal regions for *A. indica* were classified and the selection criteria for superior plants were put forward in this paper, moreover, the major contents of industry planning and technical approaches for *A. indica* plantation establishment were discussed and the countermeasures to reduce the neem-based pesticide products were also proposed.

Keywords: *Azadirachta indica*; botanical pesticide; neem plantation; product development

Introduction

Neem (*Azadirachta indica* A. Juss) is the source of unique natural products for integrated pest management, medicine, industrial and other purposes. The report of US National Academy of Sciences stated neem–tree for solving global problems (1992). It seems to be one of the most promising plants and may eventually benefit to every person on the planet. Neem has been introduced to Hainan and Guangdong provinces of China for the purpose of scientific research since 1986 (Chiu 1989). A large scale of neem plantations were established during 1999–2003 in the dry-hot valleys with tropical climate conditions along the Jinshajiang, Honghe, Nujiang, and Lancangjiang Rivers in southwest China. Neem has been accepted in these areas for its multipurpose utilization and adaptability to arid areas where revegetation is a very difficult task. A number of neem plantations have been established by local forestry departments and farmer households since 1999 (Zhang 2002). Pesticide based on neem has been used by enterprises engaging in organic agriculture, but the neem based pesticide is expensive because it is shortage of the raw material and quantity of semi-finished products depends on the import. Neem based manufacturers in China begin to pay attention to the raw material production and to make efforts to set up neem plantations in order to reduce the cost of pesticide based on neem. Growing high-yield neem plantations with high content of azadirachtin along with intensive harvesting and depulping mechanization will possibly become the most efficient ways to reduce the cost of neem pesticides in China.

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Introduction of neem to China

Twenty-four provenances of two species in genus *Azadirachta* have been introduced to China from South Asia, Southeast Asia and Africa Since 1995. One provenance is *A. siamensis* and the others are *A. indica*. These species and their provenances provide ample germplasm materials for breeding, even though the original background data have not been well-defined for most of the provenances.

Analysis on the influence of climatic factors such as temperature and rainfall on tree growth showed that *A. siamensis* could not adapt to grow in the dry-hot valleys while nearly all the provenances of *A. indica* are growing well with normal growth, blossoming and fruiting in the dry-hot valleys. The exception is that one of provenances grows slowly due to the annual death of branches, apical bud and part of bark; with a higher death rate than others.

Experiments revealed that very little differences existed in morphological, phonological characteristics, nor in the growth

rhythm, growth increment and seed yield between the introduced provenances of *A. indica* and those growing in their original location under similar site conditions. For some provenances, the contents of azadirachtin (special for azadirachtin-A in this paper) in the kernel from introduced provenances are similar to or slightly higher than their origins (Hla 1987; Schmutterer 1999). In one example, the average azadirachtin content from introduced trees was 5.5 $mg \cdot g^{-1}$, but the average content from the original plant was only 4.5 $mg \cdot g^{-1}$. This kind of finding should be explored in the further tests.

Physiology of neem

The seasonal growth rhythm of the young trees of neem is similar amongst different provenances in the dry-hot valley areas. The trees almost stop growing from November till next February, and grow rapidly from May to August, but grow slowly in March, April, September and October. The young trees could also grow rapidly from April on when there is enough water moisture in the soil. Two or three year-old young trees begin blossoming and fruiting. Florescence usually takes place from April to May and the delicate fruit will be formed five days after blossoming. Fruits mature from the mid July to the beginning of September (Peng 2002). A few of superior trees might blossom and fruit in the autumn. The trees are generally evergreen, but might defoliate in February and foliate again in March or April under arid conditions.

The pericarp turns yellow from green when the fruits mature. In one investigation, all fruits (63 000) were collected from an eight-year old tree of neem. The fruits were divided into categories of green and yellow in color of pericarp. The green fruits accounted for about 2% of the total, which were evidently smaller in size than the yellow. The comparative experiment indicated that the neem seeds obtained from either the green fruits or the yellow fruits had similar germination rates. The one thousand grain weight and the kernel to seed ration of dry seeds from green fruits were separately 4% and 6.3% lower than that of the yellow fruits, but the azadirachtin content of kernels was 10% higher than the yellow fruits. The standards of physiological maturity and the appropriate harvesting time need to be explored further.

Germination rates were different among fresh seeds treated by different methods, although kernel contents were similar. Azadirachtin content decreases slowly in storage with yellow or jade-green seeds. After 12 months of storage, seed vigor was lost entirely while merely 1/6 of azadirachtin was lost, and approximately 1/4 and 1/3 of azadirachtin were lost after 30 months and 53 months of storage, respectively. Azadirachtin content extracted from the greenish and yellow kernels that were harvested from the same tree was very similar. Kernels in color of brown and black had about 2/3 and 1/2 of the yellow and green levels, respectively.

Genetics of neem

The blooming hour was between 18:00–23:00 each day, and the most flourishing time was around 20:00. Upon blooming, pollens with about 0.04 mm in diameter were released. Almost all pollens dehydrated and turned brown in the next forenoon. A controlled pollination test showed that the self-pollination rate was very low. The test by means of Random Amplified Polymorphic DNA proved that the average outcrossing rate of *A. indica* was 96.27%, with a high confidence of 95% (interval from 93.50% to 99.05%) (Wu 2006). The result is close to the study conducted by Kundu (1999).

Some obvious differences were identified in leaf configuration among the introduced provenance plants and their filial generation plants bred from these provenances. Compared to ordinary trees, the leaves of varied filial plants are dark green in color with bilateral symmetry, smooth margin, and the leaflet stalks are longer and the florescence and shape of the flowers are also similar. No normal fruits were borne although those plants had blossomed for three years. Compound leaves with anisomerous and varied number of leaflets grew irregularly from the rhachis. The yield of seeds and the content of azadirachtin are the same as ordinary trees.

Analysis on genetic diversity of four provenances of neem was conducted by means of amplified fragment length polymorphism (AFLP). The result showed that the genetic polymorphism of the four provenances was high. Statistic study on the variation of the morphologic characters on four provenances showed that the differences in height and diameter of the stem and the length of the leaflet were the three dominant indexes, and the differences in the number of leaflets and seed size were the two secondary indexes. Both AFLP and morphologic characters analysis showed that the difference of inter-provenances was less than the difference of intra-provenances (Liu 2004).

The difference in seed yield among the individual trees of the same provenance was remarkable. Seed yield of an individual tree is generally steady, although it may vary to a certain extent in different years. Azadirachtin content among individual plants from a certain provenance is different. The average azadirachtin content is 5.5 $mg \cdot g^{-1}$, varying from the minimum of 2.7 $mg \cdot g^{-1}$ to the maximum of 8.5 $mg \cdot g^{-1}$, according to 168 seed samples harvested within a season (Zhang 2006). The variation of azadirachtin content has attracted the attention of experts engaging in neem products development (Nutan 2007).

The aspect ratio of seed is the single morphological characteristic which corresponds to azadirachtin content among the kernel to seed ration, kilo-grain weight, and the aspect ratio of seed. The bigger the aspect ratio of seed, the higher the azadirachtin content might be (Wu 2006), but there is no correlation between the autumn of fruits and the content of azadirachtin for the same tree. It has been reported that vegetative propagation can be used to reproduce *A. indica*. Apart from twig grafting, the cuttage technique was popularized for some years in China, in particular, cottage by twig cutting with one leaf and a bud. Seedlings reproduced by tissue culture were grown naturally after transplanting, but the high cost of tissue culture seedlings prevents the propagation method from being popularly practiced. Twigs of *A. indica* can be grafted on *Melia toosendan* and *M. azedarach* stocks and the grafted young trees grew faster than those repro-

duced by seeds (Zheng 2006).

Bioecology of neem

Damage from severe climate was investigated by checking the response of *A. indica* to the lowest temperature in the past 40 years in the regional test. No symptom of cold damage appeared when the lowest temperature dropped to 2°C. Some young trees were affected by low temperatures of 0-2°C, showing defoliation and leaf wilt. At the lowest temperature of 0°C or below, the tips, branches, and even stems of trees died. The longer the cold weather lasted, the worse the cold damage was. Higher humidity with low temperature made the cold damage worse. Damage to plants was strengthened by frost and ice. The older and stronger trees, the more resistance to cold damage can be (Lai 2006).

Four experimental plots were chosen to test adaptability of *A. indica* in Yunnan Province based on key factors including temperature (annual mean values from 18.3°C to 23.8°C) and rainfall (annual mean values from 611.1 mm to 1253.7 mm). The results of 10-year observation showed that the growing, blossoming and fruiting were naturally in regions with higher temperature despite the growth increment differed for corresponding to different rainfall and soil fertility conditions. *A. indica* can not grow well on the land with thin layered soil even if there is no low temperature limitation in dry-hot valleys (Peng 2003).

In Yunnan Province, *A. indica* was planted in regions where the rainfalls dropped within the range of neem's native areas, therefore temperature became the only factor that limited neem introduction according to the cold damage investigation as mentioned above. The best normal regions are areas with a minimum of temperature of 2°C. The normal region is the area where the minimum temperature drops to a range of 0–2°C, or the minimum temperature reaches below 0°C only occasionally but the minimum mean daily temperature is higher than 0°C all year round. Marginal areas of the normal region where cold damage can sometimes be found are called the secondary normal region. Elevation is an index for determining the normal region in certain areas, but the index has to be adjusted for the complex topography and climate diversity in Yunnan.

The original vegetation type in Yuanmou basin was savannah. The result of an investigation on vegetation showed that the native plant species and their numbers were not affected by neem plantations if the coverage of *A. indica* was less than 40%, but they would be affected if coverage of neem plantations was higher than 40%. The indexes of species diversity and evenness of plant species decrease with increasing neem coverage. The litter of *A. indica*r plants is beneficial to the other plant species mixed in the forest (Liu 2004).

Improvement of the forest

Superior trees were selected from provenances adapting to test regions with the characteristics of high seed yield, high azadirachtin content in the kernel, and fast growing. Superior maternal trees were identified through continuous testing and observing the growth indexes with minimum azadirachtin content over 7.0 mg·g^{-1}.

Cutting orchards have been established to popularize superior clones of *A. indica* over the short term, which provide enough vegetative propagation materials for such as cuttings, grafting scion, and explants for tissue culture.

Planting

Planting sites were selected based on results of regional trials and planting experience. First, suitable temperature and a deep soil layer must exist in the selected site. According to land types, soil texture, and slope aspects, the planting sites were divided into three grades, i.e. fine, medium, and inferior. The fine grade site is used for the establishment of high-quality, high-yield raw material plantations. The medium and the inferior sites are used for establishment of non-commercial plantations for vegetation restoration and afforestation in the barren mountainous areas. Neem trees planted on the sites close to ditches, roads, and villages are growing better than on any other types of planting site (Zhang 2005).

Seedling cultivation is easy when plentiful fresh seed source exists. However, it is very difficult to reduce seedling breeding cost in large-scale cultivation due to the low germination rates of dry seed introduced from regions far away. The following two measures can be taken to reduce seedling propagation cost by 1) sowing the primarily germinated seeds in containers; 2) transplanting the sprouting seedling from pre-germination beds into seedling containers (Peng 2001).

Seedling standards for propagation and technical specifications for planting neem have been drafted based on planting practices used to meet the demand of neem plantings in large scales. These papers were delivered to some forestry departments and institutes to review for improvement suggestions.

Development of products based on Neem

Botanical pesticide has been used in practice of agriculture for hundreds of years (He 2006). Pesticides based on neem are possibly one of the most promising products among so many kinds of botanical pesticide (Xu 2004). Scientists engaged in botanical pesticide have paid much attention to isolate and purify active substance from neem seed since the 1970s (Yamasaki 1986; Andrew 1999; Arnason 1989). Studies on efficacy test of neem insecticide started in 1968 (Butterworth and Morgan 1968) and the results confirmed that more than 400 species insect pests were susceptible to active substance from neem tree (Schmutterer 2002). During 1980s to 2000, producers of neem products mushroomed and the price of the two products viz neem oil and azadirachtin extracts varied from very low price to a very high price in the market depending on the purity and market acceptability and affordability.

Emulsifiable concentrate (EC) with 0.3% azadirachtin is the staple pesticide products based on neem in present Chinese market, which has been applied to pest management for cash crops, such as vegetables, tea-leaf, fresh fruit and coffee etc. A significant progress has been made by some enterprises and research

institutes in developing new formulae of environment protection pesticides to meet the demands of organic agriculture. Although the organic food production in China is still in the primary stage, and the application of pesticide based on neem in China only accounts for an extremely low proportion in the total pesticide consumption, 1155 enterprises, with 0.3 million ha of planting areas, had obtained the certification on organic food up to 2004. Moreover, Chinese government has already completely prohibited the use of highly toxic pesticides such as methamidophos in agriculture. With the development of organic agriculture and the restriction of high-poisoned pesticide, the pesticide based on neem will be extensive concerned and more widely used in the future (Zhang 2007).

The raw azadirachtin pesticide with 10%–40% in content, produced by Chinese enterprises, is suitable for preparation of many pesticide formulae as emulsifiable concentrate, suspension concentrate, soluble powder and microcapsule. As a by-product of nimbin pesticide, nimbin oil preparation can be made from the neem oil. Preparation techniques was developed for processing standard azadirachtin-A to 97% purity. Methods on testing the azadirachtin content in the kernel and the raw azadirachtin pesticide were established. This is the foundation to develop pesticide products based on neem. Furthermore, some daily chemical products such as Neem Moisturising Cream, Neem Shampoo, Neem Soap, Neem Toothpaste, and insect sex pheromones such as poplar clearwing moth *Paranthrene tabaniformis,* cotton leaf worm *Prodemia litura*, diamond-back moth, *Plutella xylostella,* etc. were developed for the market. The enterprises related to neem industry had reorganized to form a new combination. The dispersive financing and the power of scientific research were centralized to strengthen the investment on the development of new products and the technological innovation. It can be expected that the development and application of neem-based products can be faster and healthier in the future.

Industry planning and plantation establishment

Neem trees have been massively planted in the regions with tropical climate conditions in Hainan, Sichuan, and Yunnan provinces since 1999, driven by the implementation of 'Grain for Green' project and the Natural Forest Protection Project in China. Till 2005, 30 million neem trees had been planted on the barren lands for purpose of vegetation restoration. However, the majority of these trees can not be utilized for pesticide production because of the low productivity of the plantation sites. The neem trees planted on the preferable site conditions that are promising to become pesticide raw material plantations account for about 20% of the total. Neem plantations in China are mainly distributed in the areas with tropical climate conditions along the Honghe River, the Jinshajiang River, the Nujiang River and the Lancangjiang River in Yunnan Province. Yunnan is the first province to take the neem industry as a potential industry of forestry. Forestry Department of Yunnan Province drafted out Neem Industry Development Planning in 2006. According to the planning, the establishment of the pesticide raw material plantations is the prerequisite for the development of neem industry.

The planning will be implemented within 15 years (2006–2020) to reach the final goal of ¥4.1 billion output and 26 000 ha of neem plantations. The planning period is divided into three stages by 5 years in each stage. The construction of neem plantation and research on the integrated processing techniques are the major task in the first 5 years. Neem plantations will be achieved 20 000 ha in the year of 2010 and half of which could produce seeds. Neem plantation of 26 000 ha will be completed and 3/4 of which could produce seeds in 2016. Product development and formulation of standard of products and raw materials will be the main task of the medium stage. 26 000 ha of plantations will yield seeds by the end of last stage. A system of quality control standards on the neem products will be established (Wu 2006; Zhao 2006).

Effective approaches to reduce the product cost

Standardization of planting

Standardization of planting is the prerequisite to reduce the cost of neem resource cultivation because the superior variety breeding and high yield cultivation techniques are the important contents of planting standard for pesticide raw material plantation. It will take the advantages to improve the level of operation and management on neem plantations.

Directive breeding technology

Since 2002 we have selected seeds for neem plantations from the superior provenances. Their productivity and azadirachtin content of kernel are higher than the average level of origin area. Differentiation of economic traits, such as azadirachtin content of kernel and the seed productivity of individual trees, is remarkable even if the plants were propagated from seeds of the same tree, the same population or provenance. The selected trees featured with high azadirachtin content and seed productivity were outstanding as compared to their population. These superior characters will be passed to the regeneration plants reproduced by tissue culture, cutting, and grafting. The neem seed orchard and scion nursery have been set up in Yuanyang County of Yunnan Province, including 32 superior clones of *A. indica.* Application of superior clones to the construction of neem plantation for pesticide industry will reduce the cost of seeds production and processing. The cost of pesticide products based on neem will be controlled at a reasonable level.

Intensive harvesting and depulping mechanization

Fruits harvesting and depulping are the most labor-consuming operation in the production of neem seeds. It is hard to reduce the cost of seeds production in the traditional way of harvesting and depulping. Research on the intensive harvesting and the depulping mechanization has been launched since 2006. The results showed us that development of depulping machine and the improvement of fruits harvesting and seeds collection will reduce half of the labor in the course of seed production.

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